Indian E-Journal of Pharmaceutical Sciences 01[01] 2015
www.asdpub.com/index.php/iejps ISSN: 2454-5244 (Online)

Original Article

Preliminary Phytochemical Screening and *In Vitro* Antioxidant Activity of Ethanolic Extract of *Musa Acuminata* Linn. Flowers

Amarjeet Kaur[*1,2], Nardev Singh[1] and Mohammad Asif[2]

[1]Department of Pharmaceutical Chemistry, Shri Guru Ram Rai Institute of Technology & Sciences, Dehradun, (Uttarakhand), India
[2]Department of Pharmaceutical Chemistry, GRD (PG) Institute of Management & Technology, 248009, Dehradun, (Uttarakhand), India

***Corresponding Author**
Amarjeet Kaur
Department of Pharmaceutical Chemistry, Shri Guru Ram Rai Institute of Technology & Sciences, Dehradun, (Uttarakhand), India
E-mail: amarjeetkaur069@gmail.com

Keywords:
Medicinal plants, *Musa acuminata*, Antioxidant, Pharmacological activities,

Abstract

Medicinal plants are used in the Ayurvedic and Unani System of medicine besides use of many plants in the folk remedies. Medicinal plants are used in different countries as sources of many potent and powerful medicines. The intake of antioxidants present in food is an important health-protecting factor. Herbal compounds known by ancient medicine are of growing interest in the domain of prevention of diseases. The present study was evaluated phytochemical constituents and antioxidant activity on *Musa acuminata* Linn. flowers by using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The preliminary phytochemical analysis revealed the presence of phenols, steroids, flavonoids, saponins, tannins and glycosides. The ethanolic extract of banana flowers was showed potent antioxidant activity which was determined. The flower extract of banana showed the antioxidant activity due to the presence of polyphenols and flavonoids constituents.

1. Introduction

Free radicals can have a noxious effect on cells and it is believed that free radical damage is involved in the etiology of several diseases. The radicals are a by-product of various endogenous processes that can be stimulated by external factors, such as irradiation and xenobiotics[1]. Antioxidants protect against these radicals, and it is important to balance an enhanced radical production with a sufficient supply of antioxidants. There are two basic categories of antioxidants, namely, synthetic and natural. The most common synthetic antioxidants used in foods are compounds with phenolic structures of various degrees of substitution, whereas natural antioxidants are primarily plant phenolics and polyphenolic compounds that may occur in all the parts of plants[2]. Most of the antioxidants in use commercially such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) are synthetic[3]. Considerable progress has been made in recent years relating free radicals, especially reactive oxygen species in living cells to pathogenecity of various diseases including hepatic and vascular diseases. Efforts to discover antioxidants as useful drug candidates to combat these diseases are going on relentlessly. Numerous natural or synthetic antioxidant compounds have been tested with success in various disease models as well as in clinics. Antioxidants are now forged as the drug candidate to combat these diseases[1-4].

Figure 1: Photograph of *Musa acuminata* flower

All plants are naturally gifted with bioactive compounds which form the backbone of indigenous medicines[5]. The herbal plants are used in different medical practices such as Ayurveda, Unani and Siddha in the course of many centuries[6]. *Musa acuminata* is commonly known as Banana plant. *M. acuminata* are herbaceous plants[7]. It is one of the most widely distributed fruit in the tropical and subtropical countries [8]. According to the nutritional aspects, it is one of the world's chief food crops with a great source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds etc[9]. Every parts of the banana plant have medicinal uses. The flowers of banana are used in many kitchens as vegetables, while bracts are used as cattle feed and it is a good source of vitamin A and vitamin C. It is useful in treatment of various diseases and disorders such as bronchitis, constipation and ulcers [10].

The activities of natural antioxidants present in fruits because they possible to may reduce the level of oxidative stress and preventing free radicals from damaging proteins, DNA and lipids. Antioxidants are very effective against in the prevention of various human diseases including cancer, stroke, cardiovascular, alzhemier's disease and parkinson's disease[11,12]. Due to good food value and excellent therapeutic effect bananas are extensively consumed by the world people. Musa Species are having a number of pharmacological activities like ulcer protective activity, antiseptic, diuretic, cure cancer, immuno depressant, malaria, dysentary, heart burn, acid stomach, antioxidant activity and mutagenic effect[13,14]. Antioxidants are the molecules that inhibit the oxidation of molecule and play an important role to protect the human body against damage by reactive oxygen species (ROS) or Free radical. Natural antioxidants were known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory and vasodilatory activities[15,16]. Free radicals are reversal exist in our body either naturally or by the other factors and can be causes a many diseases like cancer, atherosclerosis, arthritis, parkinson's disease, alzheimer's disease, aging and other age related problems[17]. In the

present study ethanolic extract of powdered flowers of *Musa acuminata* Linn. were evaluated to determine preliminary phytochemicals and antioxidant activity.

2. Material and Methods

2.1 Chemicals

All chemical used for this study were high quality analytical grade reagents. The solvents used such as, ethanol, butanol, ethyl acetate, glacial acetic acid, pyridine, butanol, formic acid, were purchased from Central Drug House (CDH) Pvt, Ltd, Daryaganj, New Delhi and DPPH (1,1-Diphenyl-2-picrylhydrazyl) from Sigma Aldrich was used in this experimental work.

2.2 Plant material and Extraction

The flower of *Musa acuminata* was collected from Shri Guru Ram Rai (PG) College, Pathri Bagh, Dehradun in the month of April-2014. The *M. acuminata* flower were extracted by maceration method. The flower of *M. acuminata* was dried in shade at room temperature, pulverized and coarse powder. About 20 g of dried flower powder of the *M. acuminata* was subjected to maceration with 95% ethanol for one week. The flask was securely plugged with cotton and was shaken periodically. After complete maceration the ethanolic extract was filtered through Whatman No. 4 paper to get the clear filtrate. The ethanolic extract was evaporated under reduced pressure by using rotavapour. The ethanolic extract was used for the evaluation of phytochemical screening and antioxidant activity.

2.3 Qualitative analysis of phytochemicals constituents

The qualitative chemical tests for various Phytochemical constituents like Carbohydrates, Saponins, Flavanoids, Steroids, Glycosides, Tannins , Reducing sugar, alkaloids, anthraquinones and phenolics compound were carried out on the ethanolic extract of *Musa acuminata* Linn.

2.3.1 Detection of carbohydrates: Taken 2 ml of the extract, 2 ml of Molish's reagent and 2 ml of concentrated sulphuric acid was added. Formation of a reddish ring indicated the presence of carbohydrate.

2.3.2 Detection of reducing sugar: Taken 2 ml of Fehling's solution was added to 2 ml of the extract and boiled for 5 minutes. Formation of a brick red precipitate indicated the presence of reducing sugar.

2.3.3 Detection of alkaloids: A little of the extract was taken and stirred with Mayer's reagent (potassium mercuric iodide). Formation of cream colored precipitate indicated the presence of alkaloids.

2.3.4 Detection of saponins: Taken About 2 ml of the extract was diluted with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

2.3.5 Detection of tannins: Taken 2 ml of the extract, few drops of 1% lead acetate were added and the formation of yellowish precipitate indicated the presence of tannins.

2.3.6 Detection of flavonoids: Taken a small quantity of the extract and dilute sulphuric acid was added. The appearance of orange color indicated the presence of flavonoids.

2.3.7 Detection of terpenoids: Taken 2 ml of extract, 2 ml of acetic acid and sulphuric acid were added. Formation of bluish green ring indicated the presence of terpenoids.

2.3.8 Detection of phenol: Taken small quantity of extract and was treated with 3-4 drops of ferric chloride solution. Formation of deep blue color indicated the presence of phenol.

2.3.9 Detection of anthraquinone: Taken 2ml of ethanolic extract and 2ml of 10% NH_4OH was added. A bright pink color indicated the presence of anthraquinones [6,14].

***2.3.10 Detection of steroids*:** Chloroform solution of extract was treated with concentrated H_2SO_4 (Salkovaski test). A yellow ring is formed which tern red after one minute.

2.4 Physicochemical parameters

2.4.1 Loss on drying

Loss on drying is the loss of mass express as per cent w/w. the test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of drug, which must be eliminated as far as possible. An accurately weighed quantity of about 1 to 2 g of powdered drug was taken in a glass petridish. The powder was distributed evenly. The petridish kept open in vaccum oven and the sample was dried at a temperature between100 to 105^{0}C for 2h until a constant weight was recorded. Then it was cooled in a dessicator to room temperature, weighted and recorded. % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \text{ X } 100$$

2.4.2 Ash values

Ash values are helpful in determining the quality and purity of crude drugs, especially in the powered form. The objectives of ash value to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drug normally leaves an ash usually consisting of carbohydrates, phosphates and silicates of sodium, potassium, calcium, and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

2.4.2.1 Total Ash value

Accurately about 2 g of the powdered drug was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450^{o}c for 4 h, until free from carbon, cool and weigh. Calculated the percentage of ash with references to air-dried drug using following formula,

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \text{ X } 100$$

2.4.2.2. Acid Insoluble Ash value

Ash was boiled with 25 ml of Concentrated HCl then, filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450^{0}c for 4 h. cooled in a desiccator and weighed. Subtracted the weight the insoluble matters from the total weight of ash. Calculate the percentage of water soluble ash with reference to the air-dried drug using the following formula,

$$\% \text{ Total acid insoluble ash value} = \frac{\text{Weight of total ash- Weight of acid insoluble ash}}{\text{Weight of crude drug taken}} \text{ X } 100$$

2.5 Chromatographic evaluation:

Thin Layer Chromatography (TLC) studies were carried out for various extracts to confirm the presence of different phytoconstituents in these extracts. The retardation factor (R_f) is calculated using following formula-

$$R_f = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$

Thin layer Chromatographic profile was determined by preparing glass slides using silica gel slurry for TLC and then activated in Hot air oven. After then, by applying a drop with capillary on glass slide of extracts and it was run in suitable solvent system as mobile phase. Then with help of visualizing reagent or by iodine chamber, uv chamber R_f values for isolated constituents calculated. The table shows various solvent systems along with visualizing reagents for some phytoconstituents category.

2.6 Antioxidant activity

2.6.1 DPPH radical scavenging assay

The 40µg/ml solution of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) in ethanol was prepared and 4ml of DPPH solution was added to extract solution in ethanol at different concentration (1 to 10µg/ml). Standard solution of gallic acid was prepared at various concentrations (1 to10µg/ml) by adding 4 ml of DPPH solution. A control was prepared by adding 4ml (40µg/ml) of DPPH in ethanol and make up the volume and kept for fifteen minutes in the dark. After fifteen minutes later, the absorbance was measured at 517 nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity [18].

The capability to scavenge the DPPH radical was calculated using the following equation.

$$\% \text{ RSA} = [(A_{\text{control}} - A_{\text{test}})]/A_{\text{control}}] \times 100$$

Where, A control is the absorbance of the control reaction and a test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the Banana extract is expressed comparing with standard Gallic acid[19,20].

3. Results and Discussions

3.1 Preliminary phytochemical screening:

Preliminary phytochemical screening tests exhibited presence of various phytoconstituents of ethanolic extracts. Result indicated that the floral parts of *M. acuminata* contained carbohydrates, polyphenols, steroids, flavonoids and saponins (Table 1).

Table 1: **Preliminary phytochemical screening of alcoholic extract of *Musa acuminata***

Phytochemicals	Test performed	Observation	Inference
Alkaloids	Mayer's	Cream ppt	-
Carbohydrates	Molish's	Reddish ring	-
Reducing sugar	Fehling's	Brick red ppt	-
Saponins	Foam	Foam formation	+
Flavonoid	H_2SO_4	Orange colour	+
Steroids	H_2SO_4	Yellow ring form	+
Tannins	Lead acetate	Yellow ppt	+
Terpenoids	Acetic acid +H_2SO_4	Bluish green ring	-
Phenol	$FeCl_3$	Deep blue color	+
Anthraquinone	NH_4OH	Pink color	-

3.2 Physicochemical parameters

Various physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value and loss on drying were determined. The results of the physicochemical parameters tests carried out by the powder extracts of flower of *Musa acuminata* presented (Table 2).

3.3 Chromatographic evaluation

Various physicochemical parameters are present in *Musa acuminata*. The R_f Value of were determined and different physicoconstituent present in the extracts of flower of *Musa acuminata* and R_f value presented (Table 3).

3.4 Antioxidant activity

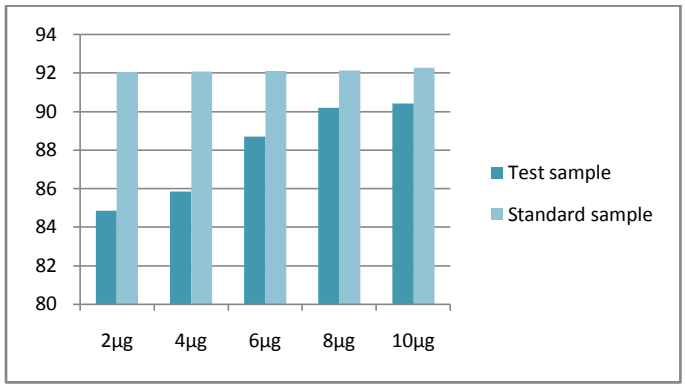
The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable organic nitrogen radical and free radical compound with a purple color which change into a stable yellow compound in reacting with an antioxidant. In brief, the reduction capacity of DPPH was determined by the decrease in its absorbance at 517 nm, which is reduced by the antioxidant (graph 1). The DPPH free radical scavenging activities of banana flower extract and gallic acid at various concentration of the extract are presented (graph 2). The addition of the flower extract into the DPPH solution caused a rapid decrease in absorbance at 517 nm indicating the excellent scavenging capacity of the banana flower extract. Result investigated the alcoholic extract of *M. acuminata* showed less antioxidant activity than standard drug gallic acid. The results indicate that the banana flowers are good sources of antioxidants including phenolics and flavonoids. Natural antioxidants that are present in *M. acuminata* are responsible for inhibiting or preventing the deleterious consequences of oxidative stress.

Table 2: Physicochemical parameters of *Musa acuminata* L.

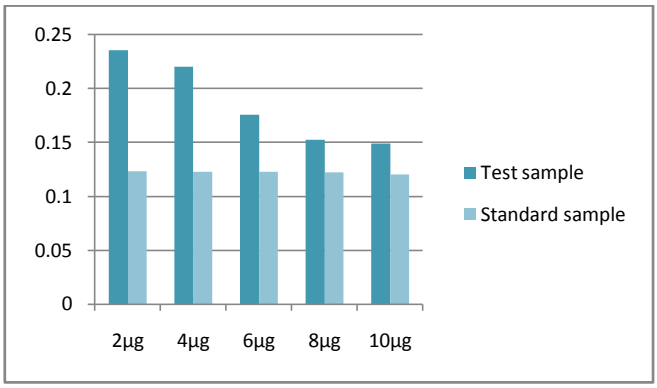
Physicochemical Parameters	Percentage (%)
Total Ash value	1.9
Acid Insoluble Ash value	1.3
Water Soluble Ash value	1.5
Loss on Drying	1.7

Table 3: R_f value of Phytoconstituents pteresent in *Musa acuminata* L.

Phytoconstituent	Solvent system	Visualizing reagents	Colour	R_f values
Alkaloids	Butanol: Glacial Acetic acid: Water. (24:24:60)	Dragendorff's reagent	Red	0.688
Flavanoid Glycosides	Ethyl Acetate: Formic acid: Glacial Acetic acid: Water. (100:11:11:27)	Polyethylene Glycol agent	Orange	0.635
Saponins	Butanol: Glacial Acetic acid: Water (50:10:40)	Ansaldehyde-H_2SO_4 reagent	Blue	0.524
Carbohydrates	Butanol: Pyridine: Water (40:24:32)	Aniline Hydrogen Phthalate reagent	Brown	0.848



Graph 1. Showing absorbance in different concentration. Gallic acid used as standard drug.



Graph 2. Showing % inhibition in different concentration. Gallic acid used as standard drug.

Free radicals released during oxidative stress pose the major endogenous damage in the biological system[21]. This type of damage is often associated with various degenerative diseases and disorders such as cancer, cardiovascular disease, immunofunction decline, and aging. Free radicals are highly reactive molecules having unpaired electrons [21-23]. They can be produced by radiation or as by-products of the metabolic process. To gain stability, free radicals capture electrons quickly from other compounds. The attacked compound becomes a free radical itself, which continues to attack other compounds and leads to a chain reaction. The disintegration of cell membranes and cell compounds, including lipid, protein, and nucleic acids. Besides damage to living cells, free radicals are the major cause of food deterioration through lipid oxidation, which ultimately affects the organoleptic properties and edibility of foods[21-23]. Hence, intervention of an antioxidant may provide therapeutic functions and maintain the freshness of food products. Recent research suggested that synthetic antioxidants could promote tumor formation as well as provide anticarcinogenic properties[22,23]. Due to these contradictory properties, the application and exploration of natural antioxidants has received more attention. Therefore, further study is required for identification and purification of antioxidant compounds in banana flowers.

4. Conclusion

This study provided evidences that ethanolic extract of *Musa Acuminata* Linn. flowers exhibited interesting direct DPPH free radical scavenging activity. The results indicate that the banana flower were good sources of antioxidant including phenolics and flavonoids. These effects may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role. It suggests that banana flowers should be considered as one of the most active substance which may positively affect human-health and can be used in food industry as an additive.

References

[1]. Vijay kumar H, Gnanendra CR, Naik N. Synthesis of Amino Acid Analogues of 5H-Dibenz[b,f]azepine and Evaluation of their Radical Scavenging Activity. *E-J Chem*, 2009; 6(1): 125-132.
[2]. Halliwell B and Gutteridge J M C, In: Hallwell B, Gutteridge J M C (Eds.), Free Radicals in Biology and Medicine, 2nd Edition. Clarendon Press, Oxford. 1989.
[3]. Allen J C and Hamilton R J, Rancidity in Foods, 2nd Ed. London, UK: *Elsevier Applied Science*, 1989.
[4]. Asif M. Chemistry and antioxidant activity of plants containing some phenolic compounds. *Chemistry International* 2015; 1(1): 35-52.
[5]. Usha V, Vijayammal PL, Kurup PA. Effect of dietary fiber from banana (*Musa paradisiaca*) on metabolism of carbohydrates in rats fed cholesterol-free diet. *Indian J. Exp. Biol.,* 1989; 27:445-449.
[6]. Horigome T, Sakaguchi E, Kishimoto C. Hypocholesterolaemic effect of banana (*Musa sapientum* L. var. cavendishii) pulp in the rat fed on a cholesterol-containing diet. *Br. J. Nutr.*, 1992; 68,231-244.
[7]. Sampath Kumar KP, Debjit Bhowmik, Duraivel S, Umadevi M. Traditional and Medicinal Uses of Banana. *J of Pharmacognosy and Phytochemistry* 2012; 1(3):51-63.
[8]. Merlene Ann Babu , Suriyakala MA, Gothandam KM. Varietal impact on Phytochemical contents and Antioxidant properties of Musa acuminata (Banana) *J Pharma Sci. & Res.*2012; 4(10):1950-1955.
[9]. Jimaima, L., Craige, T.V., Mark, W., Naiyana, W., Subramanium, S., Robert, P. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem*, 2007; 101: 1727–1741.
[10]. Gunavathy N, Padmavathy S, Murugavel SC. Phytochemical Evaluation of Musa acuminata Bracts using screening, FTIR and UV-Vis spectroscopic Analysis. *J of International Academic Research for multidisciplinary.* 2014; 2:212-221.
[11]. Shian TE, Abdullah A, Musa KH, Yusof Maskat and Ghani MA. Antioxidant properties of Three Banana Cultivars: Sains Malaysiana 2012; 41(3):319-324.
[12]. Basniwal PK, Suthar M, Rathore GS, Gupta R, Kumar V, Pareek A, Jain D. In-vitro antioxidant activity of hot aqueous extract of Helictere isora linn. Fruits. *J Natural Product Radiance.* 2009; 8(5):483-87.
[13]. Shanmugapriya K, Saravana PS, payal H, Mohammedd SP, Williams B. Antioxidant and Antimicrobial Potential of Pepper Leaves: *Indian Journal of Natural Products and Resources*.2012: 3(4): 570-577.
[14]. Victor N. Enujiugha, Justina Y. Talabi, Sunday A. Malomo, Aderonke I. Olagunju. DPPH Radical Scavenging Capacity of Phenolic Extracts from African Yam Bean (Sphenostylis stenocarpa). *Food and Nutrition Sciences*, 2012; 3: 7-13.
[15]. Badarinath AV, Rao KM, Chetty CMS, Ramkanth S, TVS Rajan, Gnanaprakash K. A Review on In-vitro Antioxidant Methods: Comparisions, Correlations and Considerations. *International J of Pharm Tech Research*. 2010; 2: 1276-1285.
[16]. Sheng ZW, Ma WH, Gao JH, Bi Y, Zhang WM, Dou HT, et.al. Antioxidant properties of banana flower of two cultivars in China: *African j of Biotechnology*. 2011; 10(21):4470-4477.
[17]. Baskar R, Shrisakthi S, Sathyapriya B, Shyampriya R, Nithya R, Poongodi P. Antioxidant Potential of Peel Extracts of Banana Varieties (*Musa sapientum*). *Food and Nutrition Sciences,* 2011; 2: 1128-1133.
[18]. Sumathy V, Lachumy SJ, Zakaria Z, Sasidharan S. In vitro Bioactivity and phytochemical screening of *Musa acuminata* flower: *Pharmacologyonline*. 2011; 2:118-127.
[19]. Nile SH, Khobragade CN. Phytochemical analysis, antioxidant and xanthine oxidase inhibitory activity of *Tephrosia purpurea* Linn. root extract: *Indian J of Natural Product and Resources* 2011; 2(1):52-8.
[20]. Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. *J Agric Food Chem* 2001; 49: 2774-2779.
[21]. Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total phenolic of edible mushroom extracts. Food Chem 2003; 81:249–55.
[22]. Kang DG, Yun CK, Lee HS. Screening and comparison of antioxidant activity of solvent extracts of herbal medicines used in Korea. *J Ethmopharmacol* 2003; 87: 231–6.
[23]. Kaur C, Kapoor HC. Antioxidant in fruits and vegetables-the millennium's health. *Int J Food Sci Technol* 2001; 36:703–25.